

Astrobiology Roadmap

Introduction

Astrobiology and the roadmap. Astrobiology is the study of the origins, evolution, distribution, and future of life in the universe. It requires fundamental concepts of life and habitable environments that will help us to recognize biospheres that might be quite different from our own. Astrobiology embraces the search for potentially inhabited planets beyond our Solar System, the exploration of Mars and the outer planets, laboratory and field investigations of the origins and early evolution of life, and studies of the potential of life to adapt to future challenges, both on Earth and in space. Interdisciplinary research is needed that combines molecular biology, ecology, planetary science, astronomy, information science, space exploration technologies, and related disciplines. The broad interdisciplinary character of astrobiology compels us to strive for the most comprehensive and inclusive understanding of biological, planetary and cosmic phenomena.

This NASA Astrobiology Roadmap outlines these multiple pathways for research and exploration and indicates how they might be prioritized and coordinated. The roadmap embodies the efforts of more than 200 scientists and technologists, including NASA employees, academic scientists whose research is partially funded by NASA grants, and many members of the broader community who have no formal association with NASA.

Fundamental questions. Astrobiology addresses three basic questions that have been asked in various ways for generations.

- How does life begin and evolve?
- Does life exist elsewhere in the universe?
- What is the future of life on Earth and beyond?

Principles. The following basic principles are fundamental to the astrobiology program:

- Astrobiology is multidisciplinary in its content and interdisciplinary in its execution. Its success depends critically upon the close coordination of diverse scientific disciplines and programs, including space missions.
- Astrobiology encourages planetary stewardship through an emphasis on protection against forward and back biological contamination and recognition of ethical issues associated with exploration.
- Astrobiology recognizes a broad societal interest in its endeavors, especially in areas such as achieving a deeper understanding of life, searching for extraterrestrial biospheres, assessing the societal implications of discovering other examples of life, and envisioning the future of life on Earth and in space.

- The intrinsic public interest in astrobiology offers a crucial opportunity to educate and inspire the next generation of scientists, technologists and informed citizens; thus a strong emphasis upon education and public outreach is essential.

Life is a central theme that unifies NASA's vision and mission. A golden age has begun for the life sciences, an age in which science and technology will benefit enormously from a fundamental understanding of the full potential of living systems. This Roadmap outlines how to achieve a better fundamental understanding both of our own world and also of potential habitable worlds and life beyond Earth. This is an agenda for inspiring the next generation of planetary explorers and stewards to sustain the NASA vision and mission.

Goals and Objectives

The NASA Astrobiology Roadmap provides guidance for research and technology development across the NASA Enterprises that encompass the space, Earth and biological sciences. The Roadmap is formulated in terms of seven Science Goals that outline key domains of investigation. For each of these goals, Science Objectives outline more specific high priority efforts for the next three to five years. These eighteen objectives will be integrated with NASA strategic planning.

Goal 1 Understand the nature and distribution of habitable environments in the Universe. Determine the potential for habitable planets beyond the Solar System, and characterize those that are observable.

A planet or planetary satellite is habitable if it can sustain life that originates there or if it sustains life that is carried to the object. The Astrobiology program seeks to expand our understanding of the most fundamental environmental requirements for habitability. However, in the near term, we must proceed with our current concepts regarding the requirements for habitability. That is, habitable environments must provide extended regions of liquid water, conditions favorable for the assembly of complex organic molecules, and energy sources to sustain metabolism. Habitability is not necessarily associated with a single specific environment; it can embrace a suite of environments that communicate through exchange of materials. The processes by which crucial biologically useful chemicals are carried to a planet and change its level of habitability can be explored through the fields of prebiotic chemistry and chemical evolution. A major long-range goal for astrobiology is to recognize habitability beyond the Solar System, independent of the presence of life, or to recognize habitability by detecting the presence of life (see Goal 7: Biosignatures).

Background. Research in astrobiology supports NASA's Origins theme in its attempt to search for habitable or inhabited environments beyond the Solar System. Humans have pondered for millenia whether other inhabitable worlds exist. Now, for the first time, they have an opportunity to look and see. Of course it is not possible to examine

the $\sim 10^{10}$ Earth-like planets that simple statistical models predict to exist in our galaxy, much less the $\sim 10^{21}$ such planets expected to be in the universe. Still, it should be possible to determine whether terrestrial planets are indeed as common as predicted above, whether a substantial fraction of them show signs of habitability, and whether an appreciable fraction of these show biosignatures.

A key difference between the search for life in the Solar System and the search in external planetary systems is that, within the Solar System, interplanetary transfer of viable microbes seems a plausible process, and therefore the discovery of life elsewhere in the Solar System seems plausible. While this is indeed of extraordinary interest, it may not cast light on whether it is easy or difficult for life to begin. On the other hand, the fact that dispersion times between stars are $\sim 10^5$ to 10^6 times longer than for dispersion within the Solar System makes independent origination of life-forms outside the Solar System more probable.

The research objectives under this goal address three key questions. First, do terrestrial planets and large satellites tend to form in a state where they are likely to become habitable, or do habitable environments emerge only after a sequence of less probable events? Second, how frequently do habitable environments arise on solid planets, including large satellites? Third, what are the specific signs of habitability and habitation, and how do such signs change with the circumstances of the planet (e.g., mass, distance from its star, history and relative abundance of volatile compounds)? To address these questions effectively, we must investigate how habitable planetary systems form and evolve (Goals 1 and 2), and we must understand the ultimate environmental limits of life (Goal 5).

Much of this effort focuses upon the presence or absence of liquid water in bulk form. Water is made from the two most abundant chemically reactive elements in the universe, and it is the necessary ingredient for Earth's type of life. Liquid water has played an intimate, if not fully understood, role in the origin and development of life on Earth. Water contributes to the dynamic properties of an Earth-sized planet, permitting convection within the planetary crust that might be essential to supporting Earth-like life by creating local chemical disequilibria that provide energy for life. Water maintains a strong polar-nonpolar dichotomy with certain organic substances. This dichotomy has allowed life on Earth to form independent stable cellular structures. Thus the primary focus of Goal 1 is concerned with planets having a liquid water boundary layer, although the focus may expand to include other planets or satellites as astrobiology matures as a discipline.

There is also a focus—though not exclusive—on molecular oxygen and ozone as biosignatures (see also Goal 7), and therefore on dealing with the interface between the understanding of the geological and biological aspects of oxygen, and the details of the spectral features that can be observed and interpreted remotely. Oxygen is a very common element that has provided Earth with its most distinctive biosignature. The chemical state of an Earth-like planet, as well as the geological activity that delivers reduced species to the surface environment, will cause virtually all of the molecular oxygen to be consumed unless it is produced rapidly (e.g., by oxygen-producing photosynthesis). Also, the relatively modest ultraviolet fluxes of many stars

prevent rapid production of oxygen from photo-dissociation of water. These factors will help to prevent the possibility of false positive detections of oxygen biosignatures.

The challenge of remotely detecting life on a planet that has not developed a biogenic source of oxygen is fraught with unknowns. What chemical species and spectral signatures should be sought? What metabolic processes might be operating? How does one guard against a false positive detection? Research that is guided both by our knowledge of Earth's early biosphere (i.e., before the rise of an oxygenated atmosphere) and by studies of alternative biological systems can help address these questions and provide guidance to astronomers seeking evidence of life elsewhere (Goal 7).

Objective 1.1 Models of formation and evolution of habitable planets. Investigate how solid planets form, acquire liquid water and other volatile species and organic compounds, and how processes in planetary systems and galaxies affect their environments and habitability. Use theoretical and observational studies of the formation and evolution of planetary systems and their habitable zones to predict where water-dependent life is likely to be found in such systems.

Example investigations

- Study the relationship between stellar metallicity and planet formation. Determine if there is a galactic habitable zone.
- Model the origin of planetary systems, especially water delivery to and loss from terrestrial-like planets of various size and mass. Determine how water loss affects climate, surface and interior processes, and how these changes affect habitability.
- Develop comprehensive models of the environments of terrestrial-like planets to investigate the evolution of habitability.

Objective 1.2 Indirect and direct astronomical observations of extrasolar habitable planets. Conduct astronomical, theoretical, and laboratory spectroscopic investigations to support planning for and interpretation of data from missions to detect and characterize extrasolar planets.

Example investigations

- Investigate novel methods for detecting and characterizing extrasolar planets, particularly those that might lead to an improved understanding of the frequency of habitable Earth-like planets.
- Use atmospheric models to understand the range of planetary conditions that can be determined from low resolution, full-disk spectra at visible, near-IR, and thermal wavelengths. Use data on Venus, Earth, and Mars to validate these models.
- Model a variety of biosignatures, including the ozone 9.7 micron band and oxygen A band signatures and their variations over Earth's geological and biological history (also relevant to Objective 7.2).

Goal 2 Explore for past or present habitable environments, prebiotic chemistry and signs of life elsewhere in our Solar System. Determine any chemical precursors of life and any ancient habitable climates in the Solar System, and characterize any extinct life, potential habitats, and any extant life on Mars and in the outer Solar System.

The exploration for habitable environments, life and/or prebiotic chemistry in the Solar System directly links basic research in astrobiology to NASA missions. Because little is presently known about habitable environments within our Solar System, the distribution and nature of potentially habitable environments should be determined on Mars, Titan, Europa, and other promising objects. As a corollary, we should understand the mechanisms of evolution of habitable environments throughout the Solar System. Although life elsewhere could have developed in ways different from life on Earth, our current knowledge of life and habitable environments serves as the starting point for our exploration strategy. Research in such widely divergent areas as planetary and Solar System evolution, the biology of extreme environments, and Precambrian paleontology has been instrumental in guiding NASA's search for evidence of life elsewhere in the Solar System. Earth-based analog studies and theoretical investigations, informed by data from previous Solar System missions, will assist astrobiologists to refine exploration strategies and scientific priorities for future missions.

Background. Understanding planetary habitability and the relationship between the occurrence of life and the evolution of planets is a primary organizing theme of NASA's Solar System Exploration Program. In the most basic sense, the strategy for the astrobiological exploration of the Solar System involves exploring for environments regarded as necessary for life to begin and/or persist, namely those having liquid water, energy sources that can sustain metabolism, conditions that promote the synthesis of complex organic molecules, and understanding the evolution of habitable environments on Solar System objects.

Advances in our understanding of the environmental limits of life on Earth have provided crucial information for refining our strategies to explore for life elsewhere in the Solar System. For example, a deep subsurface biosphere was discovered that included non-photosynthetic organisms that make organic compounds from hydrogen and other simple byproducts of aqueous weathering. This discovery has revolutionized our thinking about the potential for life on other planets like Mars or Europa, where surface conditions are fundamentally inhospitable to life. The necessity to explore the deep subsurface of other Solar System bodies has identified the need to develop robotic drilling systems that can penetrate 100's to 1000's of meters below the surface, where interior habitable zones of liquid water and a life-sustaining redox chemistry might exist. Of course, geological activity or meteorite impacts might have brought evidence of subsurface life to the surface, therefore the ability to identify and reach key sites with landers and rovers is also a high priority.

In preparing for future missions to explore for life and/or prebiotic chemistry in the Solar System, an important precursor activity for Astrobiology is to identify *in situ* instrumentation to support the search for complex organic molecules and life. As a starting point, there is a critical need for research to define unambiguous approaches to life detection over a broad range of environmental conditions that represent other planetary environments. Such research will also help address planetary protection issues, such as the effects of forward contamination of other planetary surfaces and the risks of back contamination associated with samples returned to Earth.

In pursuing the question of extraterrestrial life, humans have long held a fascination with Mars. Indeed, our robotic exploration of the red planet has provided compelling evidence for surface environments that could have supported life early in the planet's history. More recently, arguments have been made for the existence of a Martian groundwater system that could harbor an extant subsurface biota. Key questions include the following. If ever life arose on Mars, is it related to terrestrial life, or did Mars sustain an independent origin of life? If life never developed on Mars, is there a prebiotic chemical record preserved in ancient martian rock sequences that might contain clues about how life began on Earth?

Possibilities for subsurface habitable zones of liquid water have also been recognized in the outer Solar System. Induced magnetism, as well as surface geomorphology and chemistry, have provided compelling evidence for an ocean of liquid water (brine) beneath the icy crust of Europa. Similar conditions may also exist on two other Galilean satellites—Ganymede and Callisto. In addition, a complex prebiotic chemistry and zones of liquid water might exist on Titan. Some of these environments might resemble aspects of early Earth and thus they can teach us about our own origins. However, other environments could be quite different, and these might have hosted a prebiotic chemical evolution that led to an altogether different form of biology.

As the nature of the potentially habitable environments in our Solar System becomes better defined, the Astrobiology Program must interact with both observational astronomy and mission scientists to consider also the possibility of life in non-aqueous environments. Such a possibility can be explored during missions to places (like Titan) where liquid water is not predominant, and by developing the ability to recognize the biosignatures of life in non-aqueous environments.

Objective 2.1 Mars exploration. Through orbital and surface missions, explore Mars for potentially habitable environments, as evidenced by water or aqueous minerals. Study Martian meteorites to guide future Mars exploration. Develop the methods and supporting technologies for the *in situ* characterization of aqueous minerals, carbon chemistry and/or life.

Example investigations

- Target well-instrumented robotic rovers to sites of past aqueous sedimentation to analyze rocks for geochemistry, aqueous minerals, organic matter and fossil biosignatures.

- Develop flight-capable instrumentation for the unambiguous detection of biosignatures preserved in surface and subsurface rocks, soils and ices.

Objective 2.2 Outer Solar System exploration. Conduct basic research, develop instrumentation to support astrobiological exploration and provide scientific guidance for outer Solar System missions. Such missions should explore the Galilean moons Europa, Ganymede and Callisto for habitable environments where liquid water could have supported prebiotic chemical evolution or life. Explore Saturn's moon, Titan, for environments favorable for complex prebiotic synthesis or life.

Example investigations

- Explore the atmosphere and surface environments of Titan for evidence of complex organic chemistry and water, to provide a context for understanding potential habitability and prebiotic chemistry.
- Simulate the environment of Titan to aid in designing in situ missions and to interpret data returned therefrom.
- Develop astrobiology instrumentation that can survive the low temperature, high radiation environments of the surface of Europa. Use *in situ* methods to test models that predict the presence of energy sources for supporting life.

Goal 3 Understand how life emerges from cosmic and planetary precursors. Perform observational, experimental and theoretical investigations to understand the general physical and chemical principles underlying the origins of life.

How life begins remains a fundamental unsolved mystery. The origin of life on Earth is likely to represent only one pathway among many along which life can emerge. Thus the universal principles must be understood that underlie not only the origins of life on Earth, but also the possible origins of life elsewhere. These principles will be sought by determining what raw materials of life can be produced by chemical evolution in space and on planets. It should be understood how organic compounds are assembled into more complex molecular systems and the processes by which complex systems evolve those basic properties that are critical to life's origins. Such properties include capturing energy and nutrients from the environment, and manufacturing copies of key biomolecules. Clues from the biomolecular and fossil records, as well as from diverse microorganisms, should be explored in order to define better the fundamental properties of the living state.

Background. We must move beyond the circumstances of our own particular origins in order to develop a broader discipline, a "Universal Biology." Although this discipline will benefit from an understanding of the origins and limits of terrestrial life, it also requires that we define the environmental conditions and the chemical structures and reactions that could support life on other habitable planets. These may be very different than what we have learned to expect from the biology of Earth. For example,

liquid water is essential for all life on Earth, however, at least under laboratory conditions, certain chemical systems can undergo a form of replication in non-aqueous solvents. Furthermore, laboratory experiments that involve analogs of the nucleic acids, proteins, sugars, and lipids indicate that the particular molecular structures found in Earth-based life would not be essential in life forms having a genesis independent of life on Earth. The perspectives gained from such research will improve both the search for habitable environments in the Solar System and the recognition of biosignatures within those environments. The invention of translation, the creation of new metabolic pathways, the adaptation of organisms to extreme environments, and the emergence of multi-cellular life forms and other higher order functions, are all constrained by the intrinsic chemistry of the molecules that supported the particular example of life that achieved these innovations. Given this abundance of chemical opportunity, it seems likely that an expanded research effort will lead to novel molecular systems having the combination of properties that we associate with life processes. Such research will help us to understand better the link between molecular evolution and chemistry that is central to astrobiology.

Sources of organic compounds required for the origin of life. To understand how life can begin on a habitable planet such as the Earth, it is essential to know what organic compounds were likely to have been available, and how they interacted with the planetary environment. Chemical syntheses that occur within the solid crust, hydrosphere and atmosphere are potentially important sources of organic compounds, therefore they continue to be an important focus of research on this question. Prebiotic chemistry might begin in interstellar clouds. Laboratory simulations have recently demonstrated that key molecules can be synthesized in interstellar ices that are incorporated into nascent solar systems, and astronomical observations and analyses of extraterrestrial materials have shown that many compounds relevant to life processes are also present in meteorites, interplanetary dust particles and comets. It is likely that substantial amounts of such organic material were delivered to the Earth during late accretion, thereby providing organic compounds that could be directly incorporated into early forms of life or serve as a feedstock for further chemical evolution. An important research objective within this goal is to establish sources of prebiotic organic compounds and to understand their history in terms of universal processes that would take place on any newly formed planet. This will require an integrated program of pan-spectral astronomical observations, sample return missions, laboratory studies of extraterrestrial materials, and realistic laboratory simulations of inaccessible cosmic environments.

Origins and evolution of functional biomolecules. Life can be understood as a chemical system that links a common property of organic molecules—the ability to undergo spontaneous chemical transformation—with the uncommon property of synthesizing a copy of that system. This process, unique to life, allows changes in a living molecular system to be copied, thereby permitting Darwinian-like selection and evolution to occur. At the core of the life process are polymers composed of monomeric species such as amino acids, carbohydrates, and nucleotides. The pathways by which monomers were first incorporated into primitive polymers on the

early Earth remain unknown, and physical properties of the products are largely unexplored. A primary goal of research on the origin of life must be to understand better the sources and properties of primitive polymers on the early Earth, and the evolutionary pathway by which polymerization reactions of peptides and oligonucleotides became genetically linked.

Origins of energy transduction. Axiomatically, life cannot exist in an environment at thermodynamic equilibrium. If the environment were at equilibrium, then, by the second law of thermodynamics, no net chemical transformation would be possible. Thus we assume that life began in an environment that was far from thermodynamic equilibrium, so that free energy was available to drive the chemical transformations required for life processes. A fundamental question concerns the mechanisms by which this energy was captured by the earliest forms of life. The forms of available energy include light, chemical bond energy, and the energy of electron transfer reactions involving compounds with different redox potentials. It seems likely that photosynthesis appeared very early in evolutionary history, thus it is important to identify primitive pigment systems. Hydrothermal vents and other geothermal environments offer a second potential source of energy in the form of dissolved gases such as hydrogen and hydrogen sulfide, and mechanisms by which reduced gases in solution can deliver energy to living systems should be investigated. In contemporary cells, the energy present in chemical bonds is captured by metabolism, and the first forms of life must have incorporated linked chemical reactions as simple metabolic pathways. A primary research objective will be to identify mechanisms by which any of these energy sources were coupled to polymerization chemistry.

Origins of cellularity and protobiological systems. For life to begin in a natural setting such as a planetary surface there must be mechanisms that concentrate and maintain interacting molecular species in a microenvironment. From this perspective, life began as a bounded system of interacting molecules, none of which has the full property of life outside of that system. A bounded system of replicating, catalytic molecules is by definition a cell, and at some point life became cellular, either from its inception or soon thereafter. Besides separating the contents of a cell from the environment, membranes have the capacity to develop substantial ion gradients that represent a central energy source for virtually all life today. Boundary membranes also divide complex molecular mixtures into large numbers of individual structures that can undergo selective processes required to initiate biological evolution. A primary objective of research is to assemble laboratory versions of model cells. These will incorporate systems of interacting molecules within membrane-bounded environments. They will have the capability to capture energy and nutrients from the environment, grow through polymerization, and reproduce some of their polymeric components. Approaching this challenging problem will lead to a more refined definition of the living state, and will clarify the hurdles faced by self-assembled systems of organic molecules as they evolved toward the first life on the Earth.

Objective 3.1 Sources of prebiotic materials and catalysts. Characterize the cosmic and endogenous sources of matter (organic and inorganic) for potentially

habitable environments in the Solar System and in other planetary and protoplanetary systems.

Example investigations

- Trace the cosmic formation of prebiotic materials from the formation of interstellar molecules and solids through the processing of these materials to produce more complex compounds.
- Conduct laboratory experiments and simulations to provide a framework for analyzing meteorites and samples returned from asteroids and comets, and for interpreting spectra of interstellar clouds. Analyze meteorites and returned samples to understand the nature of extraterrestrial organic compounds.
- Identify the organic compounds and complexes produced under primordial planetary conditions through laboratory simulation experiments.

Objective 3.2 **Origins and evolution of functional biomolecules.** Identify multiple plausible pathways for the condensation of prebiotic monomers into polymers. Identify the potential for creating catalytic and genetic functions, and mechanisms for their assembly into more complex molecular systems having specific properties of the living state. Examine the evolution of artificial chemical systems that model processes of natural selection to understand better the molecular processes associated with prebiological evolution in the universe.

Example investigations

- Search for mechanisms of enantiomeric enhancement that introduced chirality into biological systems.
- Investigate polymers other than nucleic acids that have the potential to have been precursor molecules capable of containing genetic information.
- Investigate the RNA-catalyzed active site in ribosomes to better understand how RNA could have first evolved to mediate translation in early forms of life.

Objective 3.3 **Origins of energy transduction.** Identify prebiotic mechanisms by which available energy can be captured by molecular systems and used to drive primitive metabolism and polymerization reactions.

Example investigations

- Search for pigments that were plausible components of the prebiotic environment and have the capacity to capture and transduce light energy into chemical energy.
- Investigate redox reactions in which hydrogen serves as a source of free energy that could plausibly be available for early forms of life.
- Investigate mechanisms by which early boundary membranes could couple the energy available in ion gradients to the synthesis of high-energy compounds such as pyrophosphate.

Objective 3.4 **Origins of cellularity and protobiological systems.** Investigate both the origins of membranous boundaries on the early Earth and the associated properties of energy transduction, transport of nutrients, growth, and division. Investigate the origins and early coordination of key cellular processes such as metabolism, energy transduction, translation and transcription. Without regard to how life actually emerged on Earth, create and study artificial chemical systems that undergo mutation and natural selection in the laboratory.

Example investigations

- Determine how ionic and polar nutrients could permeate membrane boundaries to supply monomers and energy for intracellular metabolism and biosynthesis.
- Investigate polymerase reactions that can take place in membrane-bounded microenvironments, using external sources of monomers and chemical energy.
- Establish membrane bounded protein synthesis systems that incorporate ribosomes and mRNA in lipid vesicles.

Goal 4 **Understand how past life on Earth interacted with its changing planetary and Solar System environment.** Investigate the historical relationship between Earth and its biota by integrating evidence from both the geologic and biomolecular records of ancient life and its environments.

Understand how the planetary environment has influenced the evolution of life and how biological processes changed the environment. An improved knowledge of how life has altered diverse environments throughout Earth history will improve our ability to detect remnant biosignatures, even in cases where life has become extinct (see Goal 7: biosignatures). Correlations and cause-and-effect relationships should be sought between biological evolution and both long-term and episodic environmental changes. Insights that emerge from syntheses of these perspectives will guide our search for life elsewhere.

Background. A full understanding of the historical relationships between life and the environment requires a synthesis that draws from many different fields of science. For example, our knowledge of long-term environmental change is largely inferred from research in geophysics, geochemistry and sedimentology. The ongoing reconstruction of the phylogenetic tree of life and the time scale of evolution derive from morphology, fossils, and especially, information stored in the genomes of living organisms. Molecular biomarkers help to link biological evolution to past environments. Likewise, biogeochemical cycles of carbon and its redox partners oxygen, sulfur, and iron, are integral to Earth's biosphere, and their isotopic records help us understand how the biosphere evolved. Knowledge of chemistry, physics, and Solar System dynamics places constraints on Earth's history of environmental change. With these tools and methodological framework, astrobiologists can study the reciprocal interactions of organisms and their planetary environment and address

the following questions: What was the chemical and physical environment like when the earliest life (microbes) covered the Earth? Was this environment similar to the early environment of Mars? How, why, and when did the composition of the atmosphere change through time, including the step-wise increase in the oxidation state of the biosphere, and how did these changes impact Earth's biota? How did life respond to major planetary disturbances, such as bolide impacts, sudden atmospheric changes, and global glaciations, and were some disturbances caused by life? How has the planetary environment influenced the evolution of complex, multicellular, eukaryotic life, and what environmental changes were associated with the appearance of intelligent life? These and other questions are tied to this overarching goal that seeks to understand the historical interconnections between Earth and its biota to help guide our search for life elsewhere. All of this research requires a deeper understanding of evolutionary mechanisms at the levels of molecules, organisms and ecosystems (Goal 5). The results contribute directly to the identification of biosignatures (Goal 7).

Objective 4.1 Earth's early biosphere. Investigate the development of key biological processes and their environmental consequences during the early history of Earth through molecular, stratigraphic, geochemical, and paleontological studies.

Example investigations

- Examine the earliest sedimentary rocks for biosignatures, such as microfossils and chemical fossils.
- Search for biosignatures of key microorganisms and metabolic processes (e.g., photosynthesis) in rocks of Archean age.
- Analyze genomic sequence data of prokaryotes and identify correlations between lineage divergence and events in the history of the biosphere.

Objective 4.2 Foundations of complex life. Study the origins and evolution of life forms that eventually led to more complex multi-cellular biota that appear in the fossil record or exist today.

Example investigations

- Study carbon isotopes and other proxies of environmental change in Neoproterozoic rocks to better understand the history of global climatic perturbations that may have influenced the early evolution of complex life.
- Search for fossil evidence of eukaryotes in rocks of Proterozoic age to determine the morphology, ecology, and diversity of early eukaryotes.
- Analyze genomic sequence data of unicellular eukaryotes to gain insights into the early evolution of eukaryotic complexity, including the acquisition of cellular organelles.

Objective 4.3 Effects of extraterrestrial events upon the biosphere. Examine the records of the response of Earth's biosphere (both the habitable environment and biota) to extraterrestrial events, including asteroid and comet impacts.

Example investigations

- Examine the evolutionary, ecological, and taxonomic changes in Earth's biota following a known asteroid impact event.
- Investigate a known mass extinction event in the fossil record to determine whether it was caused or intensified by an extraterrestrial event, such as an impact or a nearby supernova.

Goal 5 Understand the evolutionary mechanisms and environmental limits of life. Determine the molecular, genetic, and biochemical mechanisms that control and limit evolution, metabolic diversity, and acclimatization of life.

The diversity of life on Earth today is a result of the dynamic interplay between genetic opportunity, metabolic capability and environmental challenges. For most of its existence, our habitable environment has been dominated by microorganisms and subjected to their metabolism and evolution. As a consequence of such microbial activities on a geological time scale, the physical-chemical environment on Earth has been changing, thereby determining the path of evolution of subsequent life. For example, the release of molecular oxygen by cyanobacteria as a by-product of photosynthesis as well as the colonization of the Earth's surface by metazoan life induced fundamental, global changes in the Earth's environment. The altered environment, in turn, posed novel evolutionary challenges to the organisms present, which ultimately resulted in the formation of our planet's major animal and plant species. Therefore this 'co-evolution' between organisms and their environment is apparently an inherent feature of living systems.

Life survives and sometimes thrives under what seem to be harsh conditions on Earth. For example, some microbes thrive at temperatures of 113°C. Others exist only in highly acidic environments or survive exposures to intense radiation. While all organisms are composed of nearly identical molecules, evolution has enabled such microbes to cope with this wide range of physical and chemical conditions. What are the features that enable one microbe to thrive under extreme conditions that are lethal to many others? An understanding of the tenacity and versatility of life on Earth, as well as an understanding of the molecular systems that some organisms utilize to survive such extremes, will provide a critical foundation for the search for life beyond Earth. These insights will help us to understand the molecular adaptations that define the physical and chemical limits for life on Earth. They will provide a baseline for developing predictions and hypotheses about life on other worlds.

Background. The evolution of biogeochemical processes, genomes and microbial communities has created the complexity and robustness of the modern biosphere.

However, we lack a fundamental understanding of how evolutionary forces, such as mutation, selection, and genetic drift, operate in microorganisms that act on and respond to changing microenvironments. We can examine the reciprocal interactions between biosphere and geosphere that can shape genes, genomes, organisms, and species interactions. Accordingly, we will begin to develop an understanding of the evolution of biochemical and metabolic machinery that drives the global cycles of the elements, as well as the potential and limits of such evolution. Furthermore, we must observe their coordination into genetic circuitries, and their integration into more complex biological entities, such as whole cells and microbial communities.

While co-evolution of the Earth's physical-chemical environment and its living world is dynamic and proceeds at all organismic levels, prokaryotic microorganisms have played a critical role in shaping our planet. Microbes can serve as highly advanced experimental systems for biochemical, genetic, and genomic studies. To date, over 100 microbial genomes have been sequenced. This unprecedented wealth of information, together with the experimental tools now available, provides a tremendous opportunity for experimental studies to be conducted on the evolution of microbial genes, genomes, and microbial communities. Such studies will uncover fundamental principles of molecular, cellular and community level evolution with relevance to Earth and other planets. Of specific interest is observing or simulating the evolution of those molecular properties that facilitate the metabolic coupling of the oxidation/reduction cycles of elements and the adaptation to novel environments, especially extreme environments, created by simulated perturbations. Hypothesis-driven experimentation on microbial ecosystems using single species with known genome sequences can be employed to predict environmental changes and evolutionary solutions. Such studies can be extended to defined mixed communities to study the plasticity and adaptation of the "metagenome," comprising the genomes of all members of a microbial community, when subjected to environmental changes and genetic flux. The evolved genotypes and phenotypes should be correlated to the specific changes they induce in the physical-chemical environment.

Our ongoing exploration of the Earth has led to continued discoveries of life in environments that have been previously considered uninhabitable. For example, we find thriving communities in the boiling hot springs of Yellowstone, the frozen deserts of Antarctica, the concentrated sulfuric acid in acid-mine drainages, and the ionizing radiation fields in nuclear reactors. We find some microbes that grow only in brine and require saturated salts to live and we find others that grow in the deepest parts of the oceans and require 500 to 1000 bars of hydrostatic pressure. Life has evolved strategies that allow it to survive even beyond the daunting physical and chemical limits to which it has adapted to grow. To survive, organisms can assume forms that enable them to withstand freezing, complete desiccation, starvation, high-levels of radiation exposure, and other physical or chemical challenges. Furthermore, they can survive exposure to such conditions for weeks, months, years, or even centuries. We need to identify the limits for growth and survival, and to understand the molecular mechanisms that define these limits. Biochemical studies will also reveal inherent features of biomolecules and biopolymers that define the physical-chemical limits of life under extreme conditions. Broadening our knowledge both of the range of environments on Earth that are inhabitable by microbes and of their adaptation to

these habitats will be critical for understanding how life might have established itself and survived in habitats beyond Earth.

Objective 5.1 Environment-dependent, molecular evolution in microorganisms.

Experimentally investigate and observe the evolution of genes, metabolic pathways, genomes, and microbial species. Experimentally investigate the forces and mechanisms that shape the structure, organization, and plasticity of microbial genomes. Examine how these forces control the genotype-to-phenotype relationship. Conduct environmental perturbation experiments on single microbial species to observe and quantify adaptive evolution to astrobiologically relevant environments.

Example investigations

- Experimentally observe the assembly of genes into novel metabolic pathways as an adaptive response to environmental changes.
- Examine microbial genome rearrangements, including gene deletion and acquisition processes, in response to nutrient change and physical-chemical stress.
- Investigate the diversity of genome stability in physiologically and genomically different microbes.

Objective 5.2 Co-evolution of microbial communities. Experimentally examine the metabolic and genetic interactions in microbial communities that have determined major geochemical processes and changes on Earth. Investigate how these interactions shape the evolution and maintenance of metabolic diversity in microbial communities. Investigate how novel microbial species establish and adapt into existing communities.

Example investigations

- Investigate small molecule interactions and their role in coordinating metabolic activities in mixed phototrophic/chemotrophic microbial communities.
- Examine adaptive mutations in individual microbial species of mixed communities in response to environmental perturbations.
- Examine the susceptibility of established microbial communities to invasion by foreign microbes.

Objective 5.3 Biochemical adaptation to extreme environments. Document life that survives or thrives under the most extreme conditions on Earth. Characterize and elucidate the biochemical capabilities that define the limits for cellular life. Explore the biochemical and evolutionary strategies that push the physical-chemical limits of life by reinforcing, replacing, or repairing critical biomolecules (e.g., spore formation, resting stages, protein replacement rates, or DNA repair). Characterize the structure and metabolic diversity of microbial communities in such extreme environments.

Example investigations

- Investigate the intrinsic properties and stability of critical biomolecules that allow microorganisms to survive severe freezing and thawing cycles.
- Biochemically characterize DNA-repair mechanisms that allow microorganisms to recover from radiation damage.
- Study survival strategies that might allow microbes to maintain their viability for very long periods of time (thousands to millions of years).

Goal 6 Understand the principles that will shape the future of life, both on Earth and beyond. Elucidate the drivers and effects of ecosystem change as a basis for projecting likely future changes on time scales ranging from decades to millions of years, and explore the potential for microbial life to adapt and evolve in environments beyond its planet of origin.

Life on Earth is based upon networks of biochemical reactions that interact with the crust, oceans and atmosphere to maintain a biosphere that has been remarkably resilient to environmental challenges. These networks of metabolic reactions developed within self-organized microbial ecosystems that collectively responded to environmental changes in ways that apparently stabilized the biosphere. Evolutionary biologists are working to understand how such biological and environmental processes have shaped specific ecosystems in Earth's history. However, it is far more difficult to employ such principles to formulate accurate predictions about the state of future ecosystems, especially when changes in planetary conditions are faster than the tempo of evolution. Predictions of this nature will require improved models of the biogeochemical cycling of critical elements, as these cycles represent the first-order interplay between the metabolic sequences of life and the surrounding physical world.

Viewing Earth's ecosystems in the context of astrobiology challenges us to consider how "resilient" life really is on a planetary scale, to develop mathematical representations of stabilizing feedbacks that permit the continuity of life in the face of rapidly changing physical conditions, and to understand the limits of these stabilizing feedbacks. Ideally, this consideration will provide insight into the potential impacts of physical changes at time scales ranging from seasonal and/or abrupt changes to changes that develop over millions of years.

The potential for microbial life to adapt and evolve in environments beyond its planet of origin should be assessed. Little is currently known regarding the consequences when earthly microbial life is transported into space or to other planets, where the environment is very different from that of Earth. The findings from such studies will determine whether life on Earth is strictly a local planetary phenomenon or can expand its evolutionary trajectory beyond its place of origin.

Background. Humans are increasingly perturbing Earth's biogeochemical cycles. In addition to impacting the carbon cycle, humans have doubled the natural global sulfur emissions to the atmosphere, doubled the global rate of nitrogen fixation, enhanced levels of phosphorus loading to the ocean, altered the silica cycle, and perhaps, most critically, altered the hydrological cycle. Relative to many natural perturbations, the effects of human activities have been extremely rapid. Understanding how these changes will affect planetary climate, ecosystem structure, and human habitats is an urgent research priority in which astrobiology can play an important role.

A conceptual continuum embraces the development of biogeochemical cycles, the evolution to the modern biosphere and ongoing human effects. Studies of processes over long time scales (millenia to millions of years) offer an observational context that extends and strengthens the interpretation of shorter time scale (annual to century) phenomena. While longer-term changes in Earth's ecosystems are strongly affected by processes such as tectonics and evolution, the relatively rapid rates of recent change, influenced by anthropogenic forcing, may have analogues in previous important events such as major extinctions.

A key objective for elucidating the sign of the feedbacks in biogeochemical cycles and for understanding how the cycles respond to perturbations is to develop quantitative models that incorporate the interactions between metabolic and geochemical processes. For example, how are the key biogeochemical cycles of the light elements (e.g., C, N, O, S, P, etc.) related? What constrains these cycles on time scales of years to millions of years? How are these cycles altered by rapid changes in climate? Does functional redundancy, as indicated by a great diversity within microbial ecosystems, ensure ecosystem resilience? Are specific metabolic pathways more sensitive to perturbations than others? How have the biogeochemical cycles co-evolved with Earth on time scales of millions of years? Our vision of the future will be sharpened by a retrospective view offered by such a biogeochemical model that is verified by preserved records. This effort is needed in order to expand the current focus on short-term changes and "what is happening" in order to perform more hypothesis-testing and thus address "why this is happening."

Biota that are transported beyond their planet of origin perhaps experience the ultimate environmental perturbation, one that, in most, if not all, cases, challenges their very existence. Still, understanding survival and evolution beyond the planet of origin is essential for evaluating the potential for the interplanetary transfer of viable organisms and thus the potential that any life elsewhere in the Solar System might share a common origin with life on Earth. Conditions in space and on other worlds might, in some cases, be much more extreme than those encountered by any of the habitable extreme environments on Earth. Therefore, studies of survivorship beyond Earth are an ultimate test of the resilience of Earth-originated life and thus its potential for diversification far beyond the limits of our current understanding.

Objective 6.1 Environmental changes and the cycling of elements by the biota, communities, and ecosystems. Conduct remote sensing, laboratory and field studies that relate the effects of environmental changes during Earth history to the cycling of key elements. Relate changes in elemental cycling to effects on the

structure and functioning of organisms, populations, communities, and ecosystems. Develop predictive models that integrate biogeochemical cycles with biological evolution and environmental change (both anthropogenic and non-anthropogenic, including extraterrestrial events [see Objective 4.3]).

Example investigations

- Construct biogeochemical models of ecosystems and test the models with isotopic and functional genomic analyses of the constituent parts of the ecosystems.
- Document the ecological impact of changes in climate, habitat complexity, and nutrient availability upon the structure and function of a selected ecosystem, as a guide for understanding changes that might occur over time scales ranging from abrupt events (a few years or less) to millions of years.

Objective 6.2 Adaptation and evolution of life beyond Earth. Explore the adaptation, survival and evolution of microbial life under environmental conditions that simulate conditions in space or on other potentially habitable planets. Insights into survival strategies will provide a basis for evaluating the potential for interplanetary transfer of viable microbes and also the requirements for effective planetary protection.

Example investigations

- Document the impact of the space environment upon microbial ecosystems that might be ejected into space by an impact event.
- Examine the survival, genomic alteration, and adaptation of microbial ecosystems in a simulated martian habitable environment. Interpret the significance of the findings regarding the potential for the forward biological contamination of Mars.
- Examine the effects of the space environment upon the biosynthesis and utilization of microbial biomolecules that play key roles in biogeochemical processes.

Goal 7 Determine how to recognize signatures of life on other worlds and on early Earth. Identify biosignatures that can reveal and characterize past or present life in ancient samples from Earth, extraterrestrial samples measured *in situ*, samples returned to Earth, remotely measured planetary atmospheres and surfaces, and other cosmic phenomena.

Our concepts of life and biosignatures are inextricably linked. To be useful for exploration, biosignatures must be defined in terms that can be measured and quantified. Measurable attributes of life include its complex physical and chemical structures and also its utilization of free energy and the production of biomass and wastes; phenomena that can be sustained through self-replication and evolution. Habitable planets create nonbiological features that mimic biosignatures and therefore must be understood in order to clarify our interpretations. We must create a

library of biosignatures and their nonbiological mimics of life as we know it. A strategy is needed for recognizing novel biosignatures. This strategy ultimately should accommodate a diversity of habitable conditions, biota and technologies in the universe that probably exceeds the diversity observed on Earth.

Background. Astrobiological exploration is founded upon the premise that signatures of life (biosignatures) encountered in space will be recognizable. A *biosignature* is an object, substance and/or pattern whose origin specifically requires a biological agent. The usefulness of a biosignature is determined, not only by the probability of life creating it, but also by the improbability of nonbiological processes producing it. An example of such a biosignature might be complex organic molecules and/or structures whose formation is virtually unachievable in the absence of life. A *potential biosignature* is a feature that is consistent with biological processes and that, when it is encountered, challenges the researcher to attribute it either to inanimate or to biological processes. Such detection might compel investigators to gather more data before reaching a conclusion as to the presence or absence of life.

Catalogs of biosignatures must be developed that reflect fundamental and universal characteristics of life, and thus are not restricted solely to those attributes that represent local solutions to the challenges of survival. For example, certain examples of our biosphere's specific molecular machinery, e.g., DNA and proteins, might not necessarily be mimicked by other examples of life elsewhere in the cosmos. On the other hand, basic principles of biological evolution might indeed be universal.

However, not all of the universal attributes of life will be expressed in ancient planetary materials or detectable remotely (e.g., by astronomical methods). For example, the processes of biological evolution are highly diagnostic for life, but evidence of biological evolution might *not* be readily detected as such in a sample returned from Mars. However, better-preserved evidence of life might include complex structures that are often retained in aquatic sediments or can be preserved in large quantities in the environment. Thus, for example, categories of biosignatures can include the following: cellular and extracellular morphologies, biogenic fabrics in rocks, bio-organic molecular structures, chirality, biogenic minerals, biogenic stable isotope patterns in minerals and organic compounds, atmospheric gases, and remotely detectable features on planetary surfaces (photosynthetic pigments, etc.). On Earth, biosignatures also include those key minerals, atmospheric gases and crustal reservoirs of carbon, sulfur and other elements that collectively have recorded the enduring global impact of the utilization of free energy and the production of biomass and wastes. Oxygen-producing photosynthesis has simultaneously created large reservoirs of atmospheric oxygen, marine sulfates and sedimentary ferric iron and sulfates (its oxidized products), as well as large sedimentary reservoirs of biogenic organic matter and sulfides (its corresponding reduced products). Again, such features must be sufficiently complex and/or abundant so that they retain a diagnostic expression of some of life's universal attributes. Also, their formation by nonbiological processes should be highly improbable.

As more complex biological features eventually evolved, as evidenced by plants and animals, the associated biosignatures became easier to distinguish from the abiotic

world. Human technology continues this trend, with the added benefit that it might be detected remotely. Thus, although technology is probably much more rare than life in the universe, its associated biosignatures perhaps enjoy a much higher “signal-to-noise” ratio. Accordingly, current methods should be further developed and novel methods should be identified for detecting electromagnetic radiation or other diagnostic artifacts that indicate remote technological civilizations.

Objective 7.1 Biosignatures to be sought in Solar System materials. Learn how to recognize and interpret biosignatures which, if identified in samples from ancient rocks on Earth or from other planets, can help to detect and/or characterize ancient and/or present-day life.

Example Investigations

- Determine additional organic biomarkers that will help to chart the presence and development of photosynthetic microbiota in Precambrian rocks.
- Determine the features of sedimentary laminated textures that uniquely require biological processes.
- Identify examples of chemical, mineralogical and stable isotopic biosignatures that can indicate the presence of subsurface biota (e.g., microbes living in aquifers), and that can be preserved in ancient rocks.

Objective 7.2 Biosignatures to be sought in nearby planetary systems. Learn how to measure biosignatures that can reveal the existence of past or present life through remote observations.

Example Investigations

- Determine the nature and fate of reduced gases that are produced by specific microbial ecosystems in an anoxic (“pre-oxygenated”) biosphere.
- Carry out laboratory, observational and modeling studies to separate false from true biosignatures (e.g., atmospheric O₂ in a range of planetary environments [see Objective 1.2]).